

ANALYST:		VPDES NO	
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Parameter: Fecal Coliform

Method: MPN

09/05

METHOD OF ANALYSIS:

	18TH EDITION OF STANDARD METHODS-9221C & 9221E
	EPA MICROBIOLOGICAL METHODS (1978) p. 132
	USGS B-0050-85

	Y	N
1) Is an analytical balance with a sensitivity of at least 1.0 mg used for weighing amounts of 2 grams or less? [9020 B.2.b]		
2) Is a pan balance with a sensitivity of at least 0.1 gram used for weighing amounts larger than 2 grams? [9020 B.2.b]		
3) Are water baths incubators kept covered? [9030 B.1]		
4) Is water depth in water bath incubators sufficient to immerse tubes to the upper level of the media? [9221 E.1.B]		
5) Are corrosion proof (plastic coated, stainless steel, etc.) racks used in the incubators? [9020 B.2.o]		
6) Are all reagents of ACS or equivalent grade? [9020 B.3.e]		
7) Are prepared reagents dated, labeled and initialed by the preparer? [9020 B.3.e]		
8) Is unopened dehydrated media older than 2 years discarded? [9020 B.3.h]		
9) Is media autoclaved only once? [9020 B.3.h.2]		
10) Is dehydrated media stored in tightly closed bottles in the dark, at less than 30°C, in an atmosphere of low humidity? [9050 A.1]		
11) Is opened dehydrated media discarded after 6 months? (May be kept up to one year if stored in a desiccator and remains free flowing, free of lumps.) [9020 B.3.h]		
12) Is media prepared in batches that will be used in less than 1 week? Note-Prepared media stored in screw capped containers may be stored up to 3 months. [9020 B.3.h.4]		
13) Is the pH of prepared media checked (EC media - 6.9 ± 0.2 SU, lauryl tryptose - 6.8 ± 0.2 SU) <u>after</u> sterilization? Adjustments should be made using NaOH prior to sterilization. [9221 E.1.a; 9221 B.1.a]		
14) Is media stored out of direct sunlight to prevent excessive evaporation? [9020 B.3.h.4]		
15) If liquid media is refrigerated is it incubated overnight before use? [9020 B.3.h.4]		
16) Are fermentation tubes containing gas bubbles discarded? [9050 A.1]		
17) Following rehydration is the media dispensed and sterilized within 2 hours? [9050 A.3]		
18) Is all media (except lactos broths) autoclaved at 121°C for 15 minutes and is the media removed from the autoclave promptly after pressure reaches zero. [9020 B.3.h.2]		
19) Are lactose broths autoclaved at 121° C for 12 to 15 minutes and is the maximum time the sugar broth is exposed to heat 45 minutes? [9020 B.3.h.2]		
20) Is autoclave capable of reaching 121°C within 30 minutes? [9030 B.3]		
21) If hot air sterilizing oven is used is it of sufficient size to prevent internal crowding, and constructed to give uniform and adequate temperatures of 170°C±10°C? [9030 B.2]		

22) Is glassware clean, free from cracks and chips, and stored properly? [9030 B.3.a]

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Fecal-MPN

	Y	N
23) Do pipets have error rates that do not exceed 2.5%? [9030 B.9]		
24) Is all glassware, except when in metal containers, sterilized for at least 60 minutes at a temperature of 170°C? If it can be demonstrated with recording thermometers that temperatures are uniform, 160°C is adequate. [9040]		
25) Are pipets in metal containers sterilized at 170°C for a minimum of 2 hours? [9040]		
26) Are all dilution bottles equipped with either glass stoppers or screw caps equipped with liners that do not produce toxic or bacteriostatic action upon sterilization? [9030 B.13]		
27) Are pipets sized to deliver the required volume accurately and quickly? [9030 B.9]		
28) Is sufficient room left in the sample container to facilitate mixing by shaking? [9060 A.3]		
29) Are dilutions capable of showing permit excursions? [Permit]		
30) Are samples diluted sufficiently to insure that at least one dilution series contains a fermentation tube with no bacterial growth? [Permit; 9221 C.2]		
31) Is sufficient strength lauryl tryptose broth used to prevent media dilution beyond acceptable limits? [9221 B.1.a]		
32) Are presumptive fermentation tubes incubated at $35 \pm 0.5^{\circ}\text{C}$ and examined for gas production at 24 ± 2 hours, and 48 ± 3 hours? [9221 B.1.b.2]		
33) Are all gas positive presumptive tubes inoculated into EC media for 24 ± 2 hours? [9221 E.1.b]		
34) Are all confirmatory fermentation tubes examined for gas production after 24 ± 2 hours and incubated at $44.5^{\circ}\text{C} \pm 0.2^{\circ}\text{C}$? [9221 E.1.b/c]		
35) Are sterile disposable transfer sticks or flame sterilized inoculation loops used to transfer material to the EC media? [9221 E.1.b.1]		
36) Are all procedures carried out in a manner that will neither inhibit desired bacterial growth nor cause contamination? [9060 A.3]		
37) Are samples collected in a manner that will prevent contamination? [9060 A.3]		
38) Is MPN properly calculated? [9221 C.2]		
39) Is each fermentation tube properly identified? [Permit]		
40) Is glassware tested annually for inhibitory residues or is the detergent certified by the manufacturer to be free of inhibitory effects? [9020 B.3.a.2]		
41) Are all autoclaved materials identified with heat sensitive tape? [9020 B.2.i]		
42) Are tubes sterilized prior to disposal? [Safety]		

PROBLEMS: